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6 Iodo-δ-lactone reproduces many but not all the effects of iodide

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ABSTRACT

Background: Iodide has direct effects on thyroid function. Several iodinated lipids are biosynthesized by the thyroid and they were postulated as intermediaries in the action of iodide. Among them 6 iodo-δ-lactone (IL-δ) has been identified and proposed to play a role in thyroid autoregulation.

The aim of this study was to compare the effect of iodide and IL-δ on several thyroid parameters.

Methods: Thyroid bovine follicles were incubated with the different compounds during three days.

Results: KI and IL-δ inhibited iodide uptake, total protein and Tg synthesis but only KI had an effect on NIS and Tg mRNAs levels. Both compounds inhibited Na^+/K^+ ATPase and deoxy-glucose uptake.

As PAX 8, FOXE 1 and TITF1 are involved in the regulation of thyroid specific genes their mRNA levels were measured. While iodide inhibited the expression of the first two, the expression of TITF1 was stimulated by iodide and IL-δ had no effect on these parameters.

Conclusion: These findings indicate that IL-δ reproduces some but not all the effects of excess iodide. These observations apply for higher micromolar concentrations of iodide while no such effects could be demonstrated at nanomolar iodide concentrations.

1. Introduction

Thyroid function is regulated by a variety of different signals in addition to thyrotrophic hormone (Dumont et al., 1992). Results from different laboratories have shown that iodide inhibits a number of thyroid parameters such as thyroid proliferation and function, both *in vivo* and *in vitro*. The capability of the intracellular content of iodide to modulate the gland function may be defined as thyroid autoregulation. Inhibitory actions of iodide include iodide organification (Wolff–Chaikoff effect), hormone secretion, cyclic 3′,5′-adenosine monophosphate (cAMP) generation, thyroglobulin proteolysis, glucose and aminoacid transport, protein and RNA biosynthesis, thyroid blood flow, thyroid growth, etc. (Pisarev and Gartner, 2000; Panneels et al., 2009; Gérard et al., 2009).

Because the inhibitory action of iodide, except for thyroid blood flow, is relieved by PTU and MMI it has been proposed that an intracellular and organic iodocompound, called XI, is the intermediate of its action (Van Sande et al., 1975). The nature of the putative iodocompounds involved in this autoregulatory mechanism has been the subject of extensive work. Different compounds have been proposed to be such mediators, such as an iodoprotein (Lissitzky et al., 1961) or T_3 (Juvenal et al., 1981), but their possible role remains controversial. The biosynthesis of iodolipids has been observed in the thyroid gland from several species and their participation in thyroid autoregulation has been suggested. Boeynaems and Hubbard (1980) have reported the conversion of exogenous free arachidonic acid into 5-hydroxy-6 iodo-8, 11, 14-eicosatrienoic delta lactone (IL-δ) in rat thyroid and Dugrillon et al. (1994) demonstrated that this compound is synthesized by the human gland. Pereira et al. (1990) found α-iodohexadecanal (IHDA) as the major iodolipid in horse thyroid. Both IL-δ and IHDA mimic some of the inhibitory effects of excess iodide on several thyroid parameters.

They prevent goiter growth and cAMP accumulation in rats (Pisarev et al., 1988; Thomasz et al., 2007), and in human cells and in FRTL-5 cells IL-δ inhibits cell proliferation (Dugrillon et al., 1994; Pisarev et al., 1992).

In spite of the results commented above, IL-δ could not always reproduce the effects of iodide. It was observed that IL-δ inhibited the stimulatory effect of EGF on porcine thyroid cell proliferation but did not reproduce the inhibitory effects of KI on cAMP accumulation (Dugrillon et al., 1990).

The aim of this study was to analyze whether IL-δ can reproduce the effects of iodide on the regulation of several thyroid parameters.

2. Material and methods

2.1. Cell culture

Thyroid bovine follicles were obtained by collagenase digestion. Follicles in suspension were cultured, in M-199 medium containing insulin (10 μg/ml), transferrin

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Table 1
List of oligonucleotides and conditions used for semiquantitative RT-PCR.

Primer	Primer sequence	Annealing T (°C)	Number of cycles	Genebank
NIS-F	CACCTACGAGTACCTGGA	55	38	**FJ403201**
NIS-R	GAGCCTTCAATTCCCAGA			
Tg-F	TCAATGATGCCCAGACCA	54	35	**NM_173883**
Tg-R	AGCCCAAAGGCATACAGA			
Pax 8-F	GTGGACAGGGCAGCTA	55	35	**EU548079**
Pax 8-R	GGCAACTACAGATGGTCA			
TTF1-F	GCTACTGCAACGGCAA	54	40	**EU293622**
TTF1-R	TAGCGGTGGTTCTGGAA			
TTF2-F	AGCCGCCCTACAGCTA	57	40	**EU548080**
TTF2-R	AGCCGCCCTACAGCTA			
Actin-F	GGATGCAGAAAGAGATCA	55	30	NW 001494327
Actin-R	CTAGAAGCATTTGCGGTG			

(5 μg/ml) in the presence of TSH (500 μU/ml), TSH plus KI (10^{-5} M) or TSH plus IL-δ (10^{-5} M) for three days. TSH was added in order to obtain reconstituted thyroid follicles and to maintain NIS expression (Bernier-Valentin et al., 2006).

2.2. Iodide uptake

Follicles were washed and incubated at 37 °C with 0.5 μCi carrier free Na ^{125}I (17 Ci/mg) New England Nuclear and 1 μM sodium iodide in medium M-199. After 30 min, the follicles were centrifuged at 1500 × *g* for 5 min, washed rapidly with ice-cold buffered HBSS plus 10^{-6} M KI and its radioactivity was measured. Iodide uptake was expressed as total radioactivity and referred to protein content according to Lowry method or referred to DNA (isolated using phenol–chloroform–isoamyl alcohol and spectrophotometrically quantified). Nonspecific binding of I^- was determined in the presence of $KClO_4$ (1 mM) and this value has been subtracted from the values presented. In order to measure the incorporation to proteins, total and TCA-precipitable radioactivity was determined.

2.3. ATPase activity

Follicles were homogenized in 0.25 M sucrose, 50 mM Tris–HCl buffer, pH 7.65. The homogenate was centrifuged at 12,000 × *g* for 15 min and the pellet resuspended in 1 ml of the buffer. ATPase activity was determined according to the method of Richards et al. (1978). The assays were performed at 37 °C for 60 min, in the presence and absence of 10^{-3} M ouabain. Enzyme activity was measured by the spectrophotometric determination of phosphate, released from vanadium free ATP, using the Baginski reaction (Baginski and Zak, 1960). Data correspond to the activity inhibited by ouabain.

2.4. ^{3}H-2-deoxy-glucose uptake

Glucose uptake was measured as previously described (Krawiec et al., 1991) with slight modifications. Follicles were washed with ice-cold KRH (glucose free) and incubated in the same buffer for 20 min at 37 °C, in the presence of 0.5 μCi of 2-(1,2-^{3}H)-deoxy-D-glucose (^{3}H-DOG) (30.2 Ci/mmol, New England Nuclear), 10^{-4} M deoxy-glucose. The incubations were finished by washing the follicles three times with ice-cold KRH (Krebs–Ringer–Hepes buffer), 100 mM glucose. Nonspecific uptake was determined by addition of 10^{-1} M glucose to the incubation buffer. The follicles were solubilized in 10^{-1} N NaOH. Aliquots were taken for measurement of total radioactivity and referred to DNA.

2.5. Protein and thyroglobulin synthesis

Protein and Tg synthesis was measured by incubating the follicles in 199 medium (15 μg/ml L-methionine) in the presence of 30 μCi/ml of ^{35}S-methionine (30.2 Ci/mmol, New England Nuclear). After 24 h the follicles were washed twice and lysated in buffer RIPA (0.05 M Tris–HCl pH 7.4, 0.15 M NaCl, 0.01 M EDTA, 1%, v/v Nonidet P40, 0.1%, w/v SDS, and 0.5%, v/v deoxycholate) for 1 h at 4 °C. For total protein synthesis an aliquot was precipitated with TCA and after centrifugation the radioactivity of the original aliquot and pellet were measured. For Tg synthesis lysates were centrifuged at 12,000 × *g* for 10 min, and aliquots of cleared cell lysates containing the same amount of protein were incubated with the Tg antibody for 3 h at 37 °C, followed by incubation with antirabbit secondary antibody overnight at 4 °C. The immune complexes were washed three times with Tris–HCl 0.15 M, pH = 7.4, NaCl 0.05 M buffer, eluted by boiling for 5 min in SDS gel loading buffer, and analyzed by SDS-PAGE and radioautography.

2.6. RT-PCR analysis

Extraction and purification of RNA were performed following the TRIzol method.

Following reverse transcription using the Superscript II reverse transcriptase (Invitrogen) cDNA (1 μl, 1/10) was used in each PCR reaction in a total volume of 25 μl, with specific primers for the target molecules (bovine *NIS*, *TG*, *TITF1*, *FOXE 1*, and *PAX 8*) and the PCR Master mix (Promega).

The primers used for RT-PCR, their nucleotides sequences; the annealing temperature and the number of cycles are listed in Table 1.

The PCR cycling parameters were as follows (the annealing temperatures and cycle number were optimized for each pair of primers): initial denaturation at 94 °C for 2 min (one cycle), denaturation at 94 °C for 30 s, annealing for 60 s and extension at 72 °C for 1 min, followed by a 10 min extension at 72 °C after the last cycle.

All PCR products were separated on 2% agarose gel electrophoresis and visualized with ethidium bromide. Images of the gels were analyzed by densitometry using the Image J software. The identity of PCR products was confirmed by sequencing, using a fluorescence-based dideoxy-sequencing reaction and an automated sequence analysis on an ABI 373A DNA sequencer.

2.7. Materials

All reagents were purchased from Sigma Chemical Co. (St. Louis, MO). IL-δ was synthesized and purified as already described (Pisarev et al., 1992). ^{125}I-IL-δ was synthesized using the chloramine-T method (Arroyo-Helguera et al., 2006).

2.8. Statistical analysis

Statistical analyses were performed using ANOVA followed by Student's *t* test or Student–Newman–Keuls test for multiple comparisons. Data are expressed as mean ± SEM. Differences were considered significant at $P < 0.05$.

3. Results

To determine whether IL-δ is stable during the assays, studies with ^{125}I-IL-δ were performed. After three days of incubation, iodide uptake was decreased by perchlorate and MMI. Iodide organification was strongly inhibited by MMI as it was expected. Antithyroid compounds have no effect on ^{125}I-IL-δ uptake and binding to proteins ruling out a significant deiodination of the iodolipid. These results show that IL-δ is transported inside the cells and is stable under the present experimental conditions (Table 2).

Table 3 shows that IL-δ inhibits iodide uptake in a concentration-dependent manner. IL-δ at concentrations of 10^{-5} M and 10^{-4} M reduced iodide uptake by 33% and 59% respectively. Under similar conditions KI caused the following inhibition: 27% at 10^{-6} M, 46% at

Table 2
Uptake of ^{125}I or ^{125}I-IL-δ and binding to proteins.

	^{125}I		^{125}I-IL-δ	
	Uptake	Inc. (%)	Uptake	Inc. (%)
C	28.8 ± 1.9	90 ± 2	21.7 ± 4.8	90 ± 2
MMI	1.3 ± 0.1***	13 ± 1***	23.2 ± 1.6	93 ± 3
$KClO_4$	9.7 ± 0.7***	82 ± 2	25.4 ± 1.8	89 ± 4

Follicles were preincubated for 72 h with the different compounds. ^{125}I or ^{125}I-IL-δ was present during 70 h and their uptake and binding to proteins were measured as described in Section 2 (except that there were no carriers). Uptake is expressed as cpm/μg proteins and binding to proteins is expressed as the percentage of TCA-precipitable radioactivity. Each value is the average of 4–6 experimental determinations by quadruplicate. Results are expressed as the mean ± SEM of each group.
*** $P < 0.001$ vs. control.

Table 3
Effects of different concentrations of KI and IL-δ on iodide uptake in thyroid bovine follicles.

	^{125}I cpm/min/μg DNA	% Inhibition of iodide uptake
Control	1.285 ± 83	–
IL-δ 10^{-7} M	1.386 ± 91 (n.s.)	–
IL-δ 10^{-6} M	1.151 ± 98 (n.s.)	–
IL-δ 10^{-5} M	860 ± 58**	33
IL-δ 10^{-4} M	526 ± 39**	59
KI 10^{-7} M	1.221 ± 93 (n.s.)	–
KI 10^{-6} M	939 ± 48*	27
KI 10^{-5} M	693 ± 56**	46
KI 10^{-4} M	476 ± 43**	63

Cells were preincubated for 72 h with the different compounds, and the iodide uptake was measured as described in Section 2. Each value is the average of 4–6 experimental determinations by quadruplicate. Results are expressed as the mean ± SEM of each group.
* $P < 0.05$.
** $P < 0.01$ vs. control.

10^{-5} M; while at 10^{-4} M the inhibition was 63%. For the following studies a concentration of 10^{-5} M of both compounds (IL-δ and KI) was employed. In order to determine if iodide must be organified to observe its effect, MMI, which inhibits thyroid peroxidase and iodide oxidation, is generally used. We could not use this drug in this case because we have shown that after three days of incubation with MMI, it increases inorganic iodide content which impairs its own uptake (Table 2).

To further explore the molecular mechanism involved in IL-δ effect on iodide uptake, NIS mRNA level was measured by semi-quantitative RT-PCR. Fig. 1 shows that the pre-treatment of thyroid follicles with KI caused a significant reduction (41%) on NIS mRNA levels. This inhibition is relieved by MMI (10^{-3} M) demonstrating that an organic iodocompound is required in this action. When the follicles were treated with IL-δ, no effect was observed indicating that under these conditions IL-δ exerts its effect through a nontranscriptional mechanism or independent of NIS.

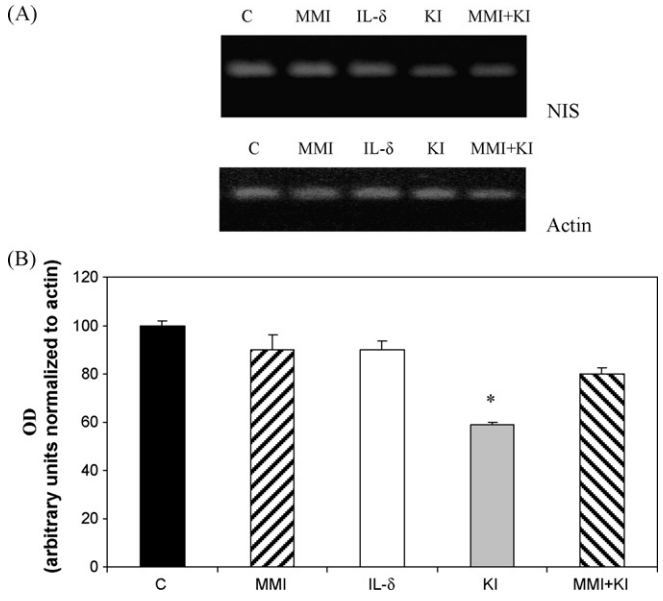

Fig. 1. Effect of iodide and IL-δ on NIS mRNA expression. Thyroid bovine follicles were preincubated for 72 h with the different compounds. Total RNA was then purified and NIS mRNA was quantified as described. (A) Representative NIS and actin RT-PCR. (B) Densitometric analysis of NIS RT-PCR. Data are expressed as the level of NIS mRNA relative to actin mRNA. Each value represents the mean ± SEM of four independent experiments. Control value was taken as 100. *$P < 0.05$ vs. TSH alone (C).

Table 4
Effects of KI (10^{-5} M) and IL-δ (10^{-5} M) on ATPase activity in thyroid bovine follicles.

	ATPase activity	% Inhibition of ATPase activity
Control	2.18 ± 0.32	
IL-δ	1.42 ± 0.18*	35
KI	1.33 ± 0.21*	39
MMI (10^{-3} M)	2.09 ± 0.38	–
MMI + IL-δ	1.38 ± 0.20*	37
MMI + KI	2.29 ± 0.020	–

ATPase activity is expressed as nmol P released/μg DNA during 60 min. Cells were preincubated for 72 h with the different compounds, and the ATPase activity was measured as described in Section 2. Each value is the average of 4–6 experimental determinations by quadruplicate. Results are expressed as the mean ± SEM of each group.
* $P < 0.05$ compared with control.

Table 5
^{3}H-DOG uptake in thyroid bovine follicles cells treated during 72 h with KI (10^{-5} M) or IL-δ (10^{-5} M).

	^{3}H-DOG uptake (dpm/μg DNA)	% Inhibition of ^{3}H-DOG Uptake
Control	145.5 ± 11.3	
IL-δ	53.8 ± 5.1*	63
KI	65.5 ± 5.8*	55
MMI (10^{-3} M)	161.5 ± 18.2	–
MMI + IL-δ	46.1 ± 3.8*	68
MMI + KI	149.9 ± 16.3	–

Cells were preincubated for 72 h with the different compounds, and the ^{3}H-DOG uptake was measured after 20 min as described in Section 2.
Each value is the average of 4–6 experimental determinations by quadruplicate. Results are expressed as the mean ± SEM of each group.
* $P < 0.05$ compared with control.

As iodide transport depends on the activity of Na^+/K^+ ATPase, the effect of KI and IL-δ on this enzyme was studied. Table 4 shows, that KI and IL-δ produced a significant decrease in the enzyme activity (35% and 39% respectively). While IL-δ exerts its effect in the presence of MMI (37% inhibition) indicating that its effect is independent of the iodide which could be released from its dehalogenation, the effect of the halogen is reversed by the presence of the antithyroid drug, showing that iodide must be organified in order to exert its effect.

It was shown that excess iodide inhibits cell membrane transport not only of iodide but other metabolites; therefore we studied the effect on ^{3}H-DOG uptake. Table 5 shows that KI and IL-δ inhibited ^{3}H-DOG uptake (55% and 63% inhibition respectively). Addition of MMI blocked iodide inhibition but failed to alter the IL-δ effect (68% inhibition).

Table 6 shows the incorporation of ^{35}S-methionine into proteins. This parameter was also inhibited by KI and IL-δ (29% and

Table 6
Effects of KI (10^{-5} M) and IL-δ (10^{-5} M) on total protein synthesis by thyroid bovine follicles.

	% Protein synthesis (dpm TCA/total dpm)	% Inhibition of protein synthesis
Control	17.3 ± 0.6	–
IL-δ	12.2 ± 0.2*	29
KI	13.0 ± 0.4*	24
MMI (10^{-3} M)	11.2 ± 1.1*	–
MMI + IL-δ	8.4 ± 0.3*,°	25 (vs. MMI)
MMI + KI	12.3 ± 0.7*	0 (vs. MMI)

Follicles were preincubated for 72 h with the different compounds; the ^{32}S-methionine uptake and incorporation into protein was measured as described in Section 2. Each value is the average of 4–6 experimental determinations by quadruplicate. Results are expressed as the mean ± SEM of each group.
° $P < 0.05$ vs. MMI.
* $P < 0.05$ vs. control.

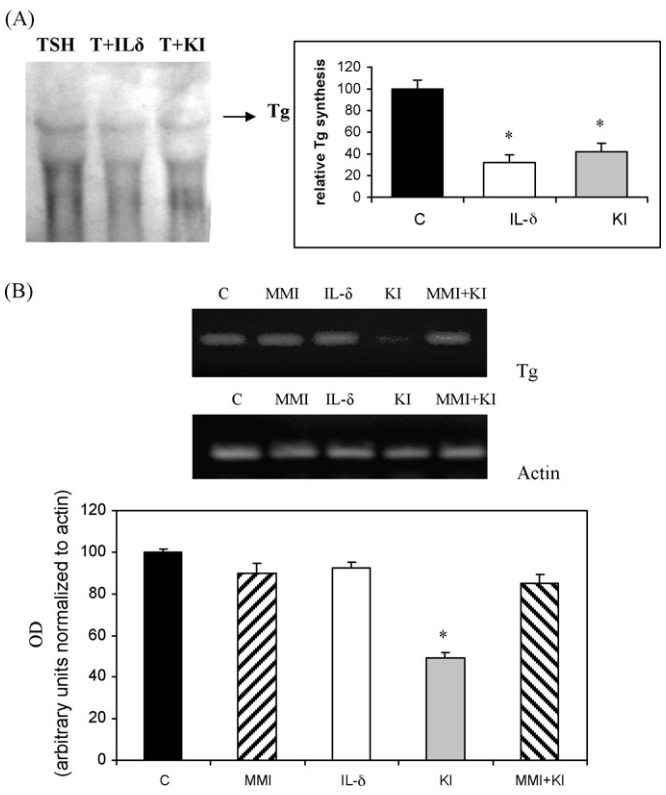

Fig. 2. Effect of KI and IL-δ on Tg protein synthesis and Tg mRNA expression. (A) Thyroid follicles were treated for 72 h as indicated and labeled with ^{35}S-methionine. Tg protein synthesis was measured incubating thyroid extracts with and an Tg antibody followed by SDS-PAGE as described. The radioautography on the right shows that KI and IL-δ inhibited Tg synthesis. On the left, the densitometric analysis, control value was taken as 100. (B) Follicles were preincubated for 72 h with the different compounds. Total RNA was then purified and Tg mRNA was quantified as described. Above, a representative Tg mRNA and actin RT-PCR. Below the densitometric analysis of Tg RT-PCR. Data are expressed as the level of Tg mRNA relative to actin mRNA. Each value represents the mean ± SEM of four independent experiments. Control value was taken as 100. *$P < 0.05$ vs. control.

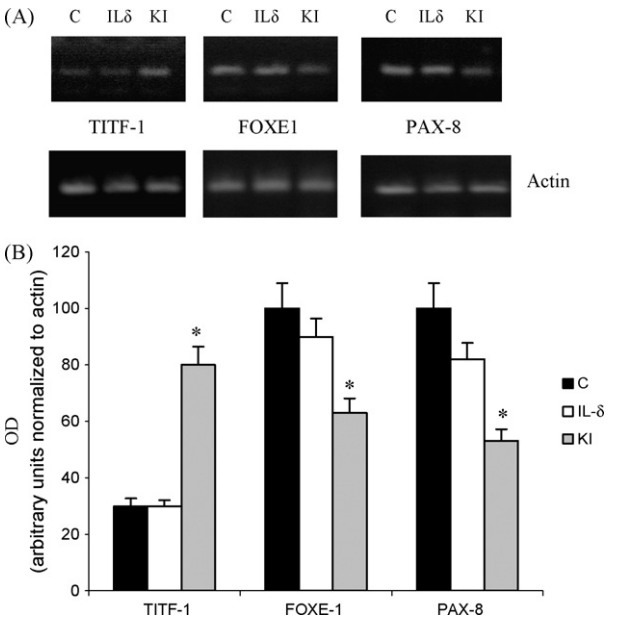

Fig. 3. Iodide effects on thyroid specific transcriptions factors (TF). Cells were treated for 72 h as described and TITF1, FOXE 1 and Pax 8 mRNAs was quantified as described in Section 2. (A) Representative TF mRNAs RT-PCR. (B) Densitometric analysis of TF RT-PCR. Data are expressed as the level of each TF mRNA relative to actin mRNA. Each value represents the mean ± SEM of at least four independent experiments. Control value was taken as 100. *$P < 0.05$ vs. C.

24% inhibition respectively). Although MMI decreased "per se" (35% inhibition) this incorporation, the effect of IL-δ could be also observed when it was incubated with the antithyroid drug. Conversely, the effect of KI was again reversed when the follicles were incubated in the presence of MMI.

The effect of KI and IL-δ on other thyroid specific parameter, thyroglobulin synthesis, was studied. As can be seen in Fig. 2(A) total protein and thyroglobulin synthesis were inhibited by KI (58%) and IL-δ (68%). But when the effect at a transcriptional level was studied only KI decreased thyroglobulin mRNA levels (Fig. 2(B)) (40%). This inhibition was again relieved by MMI.

Since transcription factors PAX 8, TITF1 and FOXE 1 are involved in the regulation of thyroid specific genes expression, their mRNA levels were measured. As can be seen in Fig. 3, iodide stimulated the expression of *TITF1* (266%) while the expression of *FOXE 1* and *PAX 8* were diminished (37% and 47% respectively). Conversely, IL-δ had no effect on these parameters, as it was expected.

4. Discussion

Iodinated derivatives from arachidonic acid, (IL-δ and omega lactone: IL-ω) inhibit several thyroid parameters such as cell proliferation (Pisarev et al., 1992; Dugrillon et al., 1990), iodide uptake (Chazenbalk et al., 1988), H_2O_2 production (Krawiec et al., 1988), goiter growth (Pisarev et al., 1988) and inositol-1,4,5-triphosphate (IP3) formation (Dugrillon and Gartner, 1995). On the other hand, α-iodohexadecanal (IHDA) inhibits NADPH oxidase (Ohayon et al., 1994), H_2O_2 production (Panneels et al., 1994a,b) and goiter growth (Thomasz et al., 2007), Regarding cAMP accumulation, in *in vitro* studies, an effect of IHDA was observed (Panneels et al., 1994a,b) but not of IL-δ (Dugrillon et al., 1990), although an inhibition on cAMP levels was observed in rats injected with IL-δ (Pisarev et al., 1988). The occurrence *in vivo* was demonstrated for IL-δ in thyroid tissue from one patient with Gravesĭ disease treated with iodide (Dugrillon et al., 1994), although the formation of IL-δ in other species could not be detected unless exogenous arachidonic acid was added (Boeynaems and Hubbard, 1980; Pereira et al., 1990). The synthesis of IHDA derived from plasmalogens, the major iodolipid formed in horse thyroid, was demonstrated by Pereira et al. (1990). This iodolipid was also detected in the thyroid of rats and dogs (Panneels et al., 1996).

Since the presence of IL-δ in thyroid is still controversial and its possible role as an intermediate of iodide action is not clear, the present studies were performed in order to clarify further if IL-δ can reproduce the effects of iodide on the regulation of several thyroid parameters.

The present data show that KI and IL-δ inhibit iodide uptake, although the action of KI is more potent at equal concentrations. To find out whether IL-δ inhibits NIS synthesis regulating mRNA levels, semiquantitative RT-PCR studies were performed. The results showed that while NIS mRNA levels are decreased by KI as it was previously demonstrated (Uyttersprot et al., 1997; Eng et al., 1999), IL-δ had no action ruling out an effect of the iodolipid at this level. MMI blocked the action of iodide confirming previous reports indicating that iodide has to be oxided and converted into an organic iodocompound in order to exert its regulatory action (Van Sande et al., 1975).

Carrier mediated transport across the cell membrane has been shown to require energy, supplied by the activity of a Na^+/K^+ ATPase. In previous results we have demonstrated that two other compounds, the free acid (14-iodo-15-hydroxy-6-eicosatrienoic acid) and its omega lactone (IL-ω) mimic the action of iodide on 2-deoxy-D-glucose and iodide uptake and this action was correlated with a decrease on Na^+/K^+ ATPase (Krawiec et al., 1991). The

synthesis of these two compounds by the thyroid has never been demonstrated, even when exogenous arachidonic acid was added. Therefore we studied the effect of IL-δ and of KI on the activity of this later enzyme. Both compounds inhibited the enzyme activity. These results indicate that while the halogen exerts its inhibitory action on iodide uptake at the membrane regulating Na^+/K^+ ATPase and NIS mRNA levels, thus confirming previous reports (Uyttersprot et al., 1997; Eng et al., 1999), the inhibitory action of the iodolipid is a more generalized effect involving the membrane level. Moreover the transport of ^{3}H-DOG was impaired by KI and by IL-δ. An action at the protein level e.g. protein degradation, protein trafficking, etc., could not be ruled out (Eng et al., 2001; Bizhanova and Kopp, 2009).

The possibility that the results obtained with IL-δ may be due to a release of iodide originated by deiodination of the iodolactone may be ruled out since MMI failed to alter the iodolipid inhibitory action on ^{3}H-DOG uptake. Moreover, experiments with IL-δ labeled with ^{125}I showed that IL-δ is transported inside the cells and is not dehalogenated during the time of assay.

The effect of KI and IL-δ on other thyroid specific protein, thyroglobulin, was also determined. Total protein was inhibited by KI and IL-δ; MMI had also an inhibitory effect "per se". It was observed that MMI inhibited TSH-stimulated cAMP production in FRTL-5 cells (Korytkowski and Cooper, 1992) and these may be the explanation of our results. Regarding thyroglobulin synthesis, it was inhibited by both KI and IL-δ. But only the former decreased Tg mRNA levels indicating once more that KI and IL-δ, in the present model, act through different mechanisms of action. Again the effect of iodide was reversed by the addition of MMI indicating that an organic iodocompound is involved in its action.

A decrease in Tg mRNA levels by iodide contrasts with previous results from our lab (Pregliasco et al., 1996) and those of Uyttersprot et al. (1997) which has not found inhibition by KI at the transcriptional level. The discrepancy may be attributed at the different models employed, rat monolayer vs. isolated follicles, or to species-specific differences in *Tg* gene regulation.

As the thyroid transcription factors, PAX 8, TITF1 and FOXE 1, are involved in the regulation of the expression of genes for several thyroid specific proteins (Damante et al., 2001; Di Palma et al., 2009), the action of IL-δ and iodide on their mRNA levels were determined. Iodide inhibited the expression of *PAX 8* and *FOXE 1*. On the contrary the expression of *TITF1* was stimulated by iodide. Although TSH stimulates the transcription of Tg, NIS, TPO mRNAs its role in the regulation of the expression of thyroid specific transcriptions factors is still unclear. While the expression of *PAX 8* and *FOXE 1* is stimulated by TSH/cAMP (Van Renterghem et al., 1995; Ortiz et al., 1997), the effect on *TITF1* seems to be different. In FRTL-5 cells its expression is down-regulated by the addition of either TSH or forskolin (Shimura et al., 1994) while in primary cultures of dog thyrocytes, there is no effect of forskolin on *TITF1* mRNA levels (Van Renterghem et al., 1996). Moreover in FRTL-5 cell line stably expressing constitutively different levels of *Ras*, *Pax 8* and *Foxe 1* were markedly down-regulated, whereas *Titf1* was much less affected, (De Vita et al., 2005). This could explain the different action of iodide on the regulation of these transcription factors. This paper provides the first evidence that physiological (micromolar) concentrations of iodide mediate the regulation of thyroid specific transcriptions factors. On the other hand IL-δ was without effect, as it was expected.

This is not the first time that it is observed that IL-δ cannot reproduce all the effects of iodide. As it was mentioned, Dugrillon et al. (1990) observed that IL-δ inhibited the stimulatory effect of EGF on porcine thyroid cell proliferation but did not reproduce the inhibitory effects of KI on cAMP accumulation. Moreover since excess iodine increases TGF-β1 mRNA expression in sheep thyroid cells (Yuasa et al., 1992) and its protein synthesis in porcine thyroid cells cultured in monolayer (Cowin et al., 1992) it was expected that iodolactone could also induce TGF-β1 biosynthesis. However in porcine follicles (Gartner et al., 1997) and in *in vivo* studies in rats IL-δ did not regulate TGF-β1 synthesis, while KI increased its expression (Thomasz et al., unpublished results).

Tg and NIS expression are regulated by TSH through cAMP cascade (Dumont et al., 1992) and the fact that IL-δ inhibits IP3 formation but not cAMP (Dugrillon et al., 1990) could explain the results herein reported.

In summary the present results provide evidence that iodide inhibits several thyroid parameters including the expression of thyroid specific transcription factors mRNAs. These observations apply for higher micromolar concentrations of iodide while no such effects could be demonstrated at nanomolar iodide concentrations. IL-δ could not reproduce these effects, at least in the model herein employed. Although these results do not support a possible role as an intermediary of the iodide action in the autoregulatory process it cannot be excluded definitively since the identity of XI has not been yet established and the possibility that more than one compound participate in this process cannot be ruled out.

Conflict of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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